with the fact that he could link the structure to speculation that cells must have a cytoskeleton that determined their shape (Needham, 1942).

Altering the Specimen to Survive Microscopy and Generate an Image

Beyond preparing a sufficiently thin specimen, electron microscopy required transforming the specimen into a condition that could both withstand the conditions in the electron microscope and produce a distinct image. In the electron microscope, specimens are subjected to vacuum, or near vacuum, conditions. The reason is that the electron microscope image is created by collisions of the electron beam with molecules, and collisions with air molecules would result in a haze in the final image. Unless researchers removed the major constituent of a cell – its water – prior to placing the cell in vacuum conditions, the water would vaporize and disrupt cell structures as it escaped. The removal of water could both shrink and distort the shape of the specimen and move or remove other cell constituents, raising serious risks of generating artifacts. In addition to dehydrating the cell, it was necessary to stabilize the structure in the cell to preserve the morphology against disruption resulting from either the removal of water or metabolic processes. This was most commonly achieved through fixation, a chemical treatment that both displaces the water and creates new chemical bonds that stabilize structures in the cell. Finally, because the resulting image was due to scattering of electrons, and because cellular materials are all of roughly the same density and will therefore scatter electrons roughly equally, it was necessary to stain those structures so that they would scatter more electrons. The chemical bonds created by many fixatives, though, also enhance scattering of electrons and thus served as a stain for electron microscopy. Thus, fixation was the central process in preparing the specimen for electron microscopy.

Because fixation involves the displacement of the water[30] and the creation of new chemical bonds in the specimen, the process radically alters conditions in the cell. The charge of artifact accompanied the use of fixation since its introduction in the late nineteenth century. These charges were fueled by the investigations of Fischer (1899), who applied different fixatives to homogenous solutions of proteins such as albumose, gelatin, egg albumin, and peptone, and generated structures that were filamentous, reticular, or granular and resembled structures observed in fixed cells. The charge of artifact was further encouraged by the fact that the same item had a different

[30] A major concern here was the fact that the fixative had to spread through the cell, creating currents which could displace soluble material or extract it from the cell altogether upon washing.